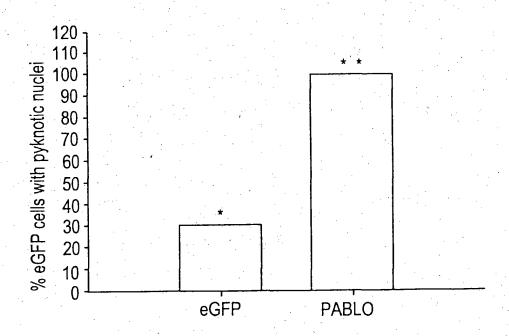
FIG. 1



Inventors: Robert John MARK, et al.
Attorney Docket No.: AM100012-P2
Title: PABLO, A POLYPEPTIDE THAT INTERACTS....

FIG. 2

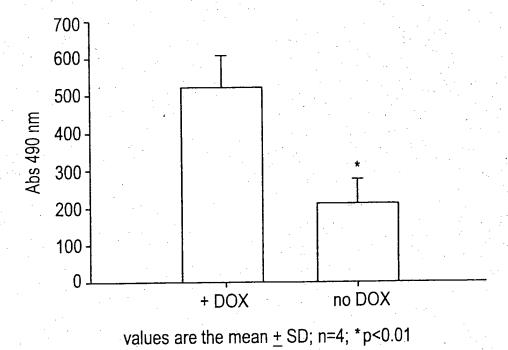


FIG. 3

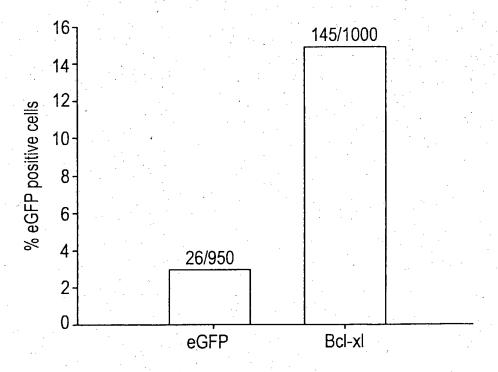


FIG. 4

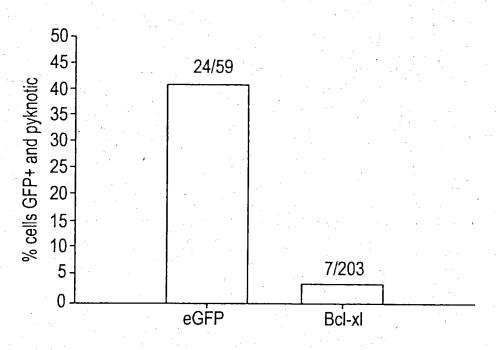


FIG. 5

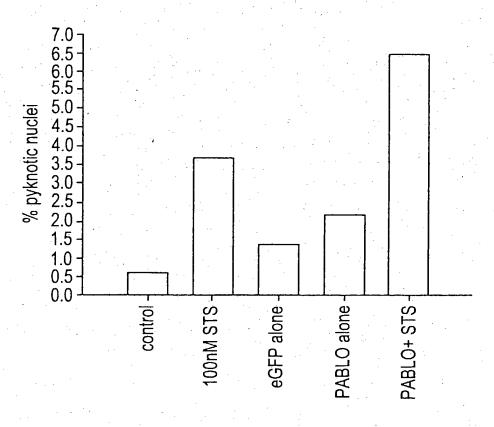
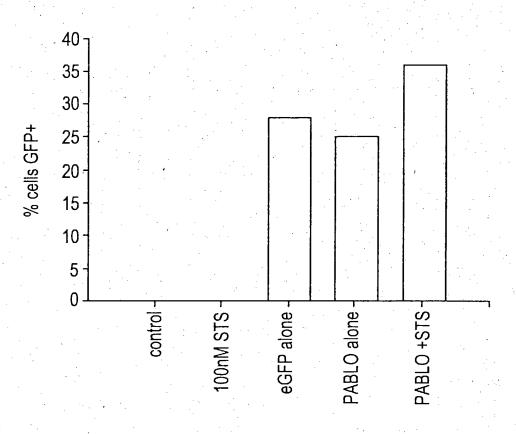


FIG. 6



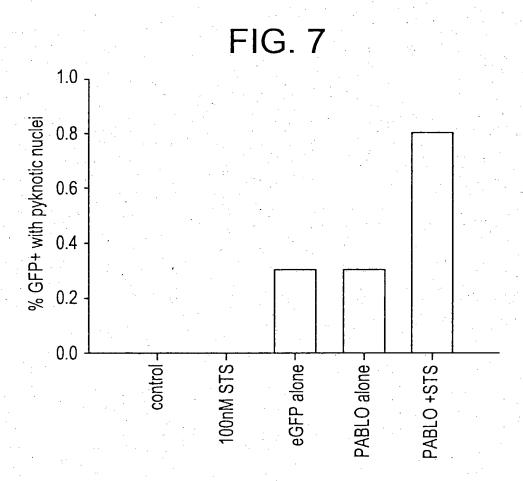


FIG. 8A

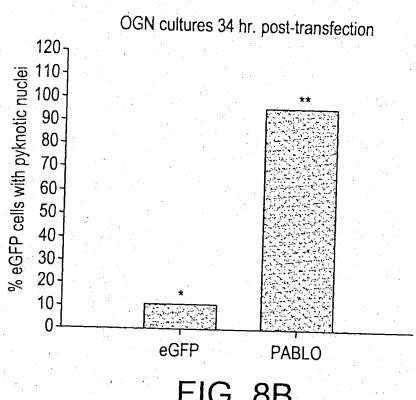


FIG. 8B

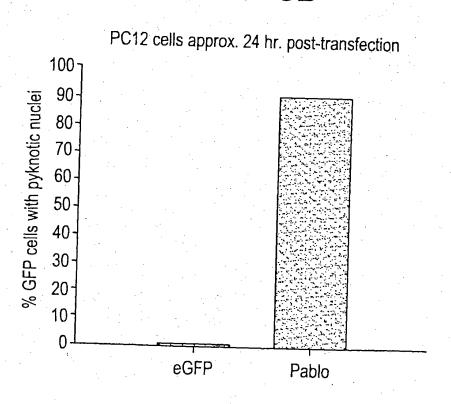


FIG. 8C

rat hippocampal cultures 30 hr. post-transfection

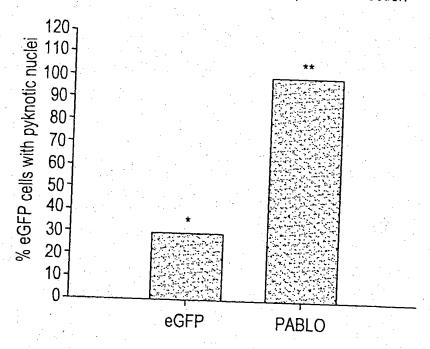
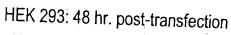


FIG. 8D



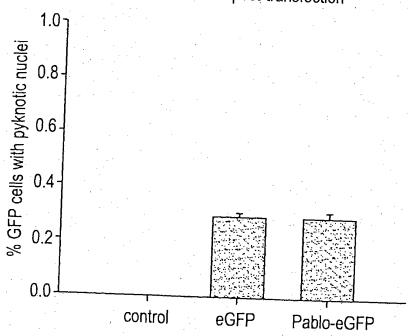
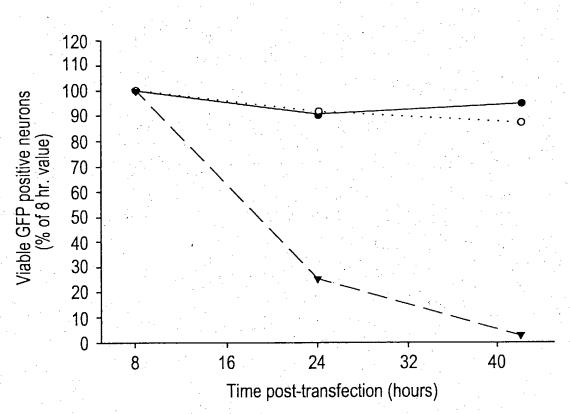


FIG. 9



- eGFP empty vectoro delta 142-eGFPfull length Pablo-eGFP

FIG. 10A Bclx1 (ATM)/PAS-1

	<u>, , , , , , , , , , , , , , , , , , , </u>	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	 	
50	GCTGGTGGTT	GGAGTCAGTT 150	ACTGAATCGG 200	GCACCTGGCA 250	GTTTGGATGC
40	GCAACCGGGA 90	TTCCCAGAAA GGATACAGCT	CCCAGAAGGG ACTGAATCGG	ACCCATCCTG	CACAGCAGCA
30	ATGTCTCAGA 80		GAAGAGAACA GGACTGAGGC 170 180	ATCAATGGCA	AGCCACTGGC
20	TCATATGAAA 70	CCTACAAGCT		CCCCAGTGCC	CGGTGAATGG
10	CAGCTTTGAC	GACTTTCTCT 110	TAGTGATGTG	AGATGGAGAC 210	GACAGCCCCG
	19 Bcl×1/pAS2- 1	19 Bcl×1/pAS2-1	19 Bclxl/pAS2- 1	19 Bclxl/pAS2- 1	19 Bclx1/pAS2- 1

			<u> </u>	· ·					
300	AGGGAGGCAG	350	CCTGACATCC	300	AACAGGTAGT	450	GTGGCCTTTT	500	GGAGATGCAG
290	GCAAGCGCTG	340	CATTCAGTGA	390	CAGAGCTTTG	440	GGGTCGCATT	490	GCGTAGACAA
280	CAGCAGTAAA	330	TACCGGCGGG	380	GACAGCATAT	430	GGGTAAACTG	480	тесетесяда
270	ATCCCCATGG	320	TGAACTGCGG	370	TCACCCCAGG	420	TTCCGGGATG	470	CGGGGCACTG
260	CCGGGAGGTG	310	GCGACGAGTT	360	CAGCTCCACA	410	GAATGAACTC	460	TCTCCTTCGG
	19 Bclx1/pAS2- 1		19 Bclx1/pAS2- 1		19 Bclx1/pAS2- 1		19 Bclx1/pAS2- 1		19 Bclx1/pAS2- 1

FIG. 10C

			-						•
550	GGAATGACCA	600	TTTGTGGAAC	650	GGAACGCTTC	700	ATTTCTTATG	750	AGTGTAT
540	GCCACTTACC	590	CTGGGATACT	640	GAAAGGGCCA	069	GCCAAGCTAA TTCCGGGCGA	740	TTTATTA AATAAGTTAT AAAAAAATA
530	AGCTTGGATG	580	TGGATCCAGG AGAACGGCGG	(930	GCCGAGAGCC	089		730	AATAAGTTAT
520	GTCGGATCGC	570	TGGATCCAGG	620	CAATGCAGCA	670	TCGACCTGCA	720	тттаттатта
510	GTATTGGTGA	560	CCTAGAGCCT	610	TCTATGGGAA	099	AACCGCTGAG	710	ATTTATGATT
	Bclx1/pAS2-		Bclx1/pAS2-		Bclx1/pAS2-		Bclxl/pAS2-		Bclx1/pAS2-
	19		19		19		61 4		1 19

FIG. 11

Bait In Yeast 2-Hybrid Screen Amino Acid Sequence of Bclxl (TM) Used As

FIG. 12A
Nucleotide Sequence of Pablo D142

10	20	30	40	20	09	70	
atgccgctagtgaaaagaaacaatgaacca	lagaaacatcgat taaccaatattt	cctaggcac	ttgtgccaca atataattag	cagcactg	atcgatcctaggcacttgtgccacacagcactgcctagaggcattaaga	taaga aaata	70
tgctgaagatatatttggaga	tggagaattatt	caatgaage	acatagttt	tccttcag	attattcaatgaagcacatagtttttccttcagagtcaactcattgcaa	tgcaa	210
gaacgtgtggaccgtttatct	ttatctgttagt	gttacacag	cttgatccaa	aggaagaa	gttagtgttacacagcttgatccaaaggaagaagaattgtctttgcaag	gcaag	280
atataacaatgaggaaagctt	aagctttccgaa	ıgttctacaa	ttcaagacca	gcagcttt	tocgaagttotacaattcaagaccagcagcttttcgatcgcaagacttt	acttt	350
360	370	380	390	400	410	420	
gcctattccattacaggagac	ıggagacg tacga	tgtttgtga	acagcctcca	cctctcaa	gtacgatgtttgtgaacagcctccacctctcaatatactcactc	cttat	420
agagatgatggtaaagaaggt		fttttatacc	aatccttcgt	atttottt	ctgaagttttataccaatccttcgtatttctttgatctatggaaagaaa	agaaa	490
aaatgttgcaagatacagagg	ıcagaggataaga	ıggaaggaaa	agaggaagca	gaagcaga	a taagaggaaggaaagaggaagcagaagcagaaaaa totaga tog too	cgtcc	φ (
tcatgaaccagaaaaagtgcc ccagagctggctgaagatgat	lagtgccaagagc Igatgatgctaat	acctcatga ctcttacat	caggcggcga aagcatattg	gaatggca aagttgct	aagagcacctcatgacaggcggcgagaatggcagaagctggcccaaggt gctaatctcttacataagcatattgaagttqctaatggcccagcctctc	aaggt ctctc	700
) 			
710	720	730	740	750	760	770	
attttgaaacaagacctcaga		tggatcata	tggatggatc	ttactcac	catacgtggatcatatggatggatcttactcactttctgccttgccatt	ccatt	770
tagtcagatgagttagcttct	Igcttctgactag	Jagotgagga	aagggtatta	gtcagacc	gactagagctgaggaaagggtattagtcagaccacatgaaccacctcca	ctcca	840
cctccaccaatgcatggagca	ggagcaggagat	gcaaaaccg	atacccacct	gtatcagt	ggagatgcaaaaccgatacccacctgtatcagttctgctacaggtttga	tttga	910
tagaaaatcgccctcagtcac		ıcaggcagaa	cacctgtgtt	tgtgagcc	cagetacaggeagaacacetgtgtttgtgageeeeaeteeeeactee	actec	980
tecaccacetettecatetge	atctgccttgtc	aacttcctc	attaagagct	tcaatgac	cttgtcaacttcctcattaagagcttcaatgacttcaactcctcccct	acct	1050

060 1070 1080 1090 1100 1110 1120	setececeacetecacetecagecaetgetttgcaagetecageagtaceacetecagete 1120	gatt
ιo l	agtacctcccc	þ

FIG. 13

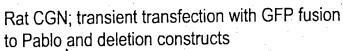
Amino Acid Sequence of Pablo Δ 142

	10	20	. 30	40	
MPLVKI	RNIDPRHLC	HTALPRGIK	NELECVTNI	SLANIIRQ	40
LSSLSI	KYAEDIFGE	LFNEAHSFS	FRVNSLQER	VDRLSVSV	80
TQLDP	KEEELSLQD	ITMRKAFRS	STIQDQQLF	DRKTLPIP	120
LQETY	DVCEQPPPL	NILTPYRDD	GKEGLKFYT	NPSYFFDL	160
WKEKM	LQDTEDKRK	EKRKQKQKN	LDRPHEPEK	VPRAPHDR	200
	.* .				
		*	• •	· 4	
	210	220	230	240	
DDEMO	ZI NOCDEI N			CHERMORO	0.40
	-		HIEVANGPA		240
		_	LTRAEERVL		280
PPPMH	GAGDAKPIP	TCISSATGL	IENRPQSPA	TGRTPVFV	320
SPTPPI	PPPPPLPSA	LSTSSLRAS	MTSTPPPPV	PPPPPPPA	360
TALQA	PAVPPPPAP:	LQIAPGVLH	PAPPPIAPP	LVQPSPPV	4,00
*	410	420	430	440	
					
ARAAP	VCETVPVHP	LPQG			418

FIG. 14
PABLO and deletion constructs

Δ70 YES В \Box \Box \Box \Box DOM. NEG. Δ142 NO ش PABLO YES В ٠مممم Toxicity

FIG. 15



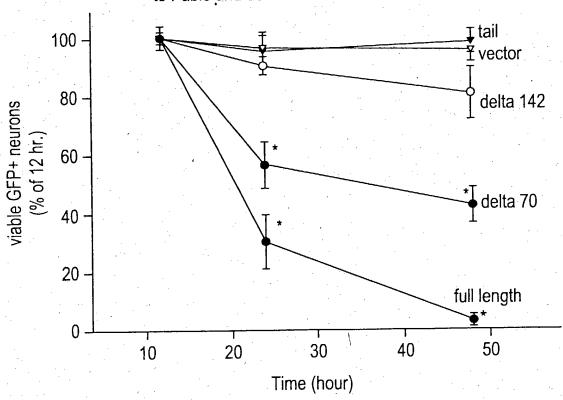


FIG. 16

PC12. Pablo plus deletion constructs

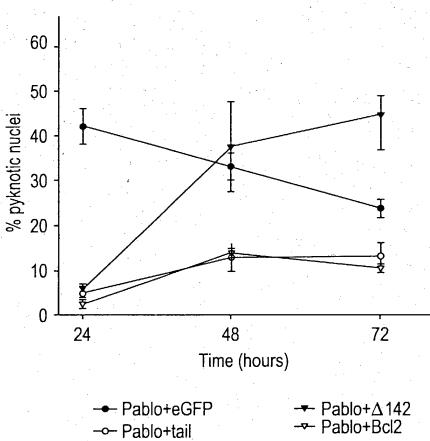


FIG. 17

Search for a Dominant Negative Pablo

Transfection	<u>DNA</u>
· 1 ··	Pablo-eGFP + eGFP
2	Pablo-eGFP + tail-eGFP
3	Pablo-eGFP + delta 142-eGFP
4	Pablo-eGFP + Bcl-2-eGFP